Supporting Information

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SI Experimental Procedures

Electron Microscopy. Single colonies were fixed in Karnovsky's fixative at 4 °C overnight. The colonies, placed in a round bottom 96-well plate under direct visualization of a microscope to facilitate rinsing without losing them, were washed three times with cacodylate buffer (1). Colonies were transferred to an Eppendorf tube, incubated with 1% osmium tetroxide in 0.1 M cacodylate buffer for 30 min, washed three times, dehydrated, embedded in eponate, and processed for transmission electron microscopy.

Immunostaining. Colonies were manually picked and fixed in 4% paraformaldehyde at 4 °C overnight. For frozen sections, colonies were cryoprotected in 30% sucrose in PBS, then embedded in Optimal Cutting Temperature compound (Sakura Finetek), and sections were cut at 10 μm. For whole-mount immunohistochemistry, colonies were incubated with blocking buffer containing 5% donkey serum and 0.1% triton at 4 °C overnight. Primary antibodies used were as listed in Table S2 and were detected with donkey-raised secondary antibodies conjugated to cyanine (Cy) 3, Cy5, DyLight488 (Jackson Immunoresearch), or Alexa488 (Invitrogen) at 1:2,000 dilution (1:500 for Cy5). ApoTome images were captured on a Zeiss Axio Observer Z1 microscope with Zeiss AxioVision 4.8 and figures prepared with Adobe Photoshop/Illustrator CS3. Projection of z-stacks to 3D images or movies was performed with Axiovision 4.8.

In Vitro Glucose Challenge Assay. Endocrine/Acinar colonies (100/well × 3 wells) were handpicked, pooled, and incubated overnight with Krebs–Ringer Bicarbonate HEPES solution (KRBH; 129 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 5 mM $NaHCO_3$, 10 mM Hepes, 0.1% BSA) containing 10% fetal calf serum (FCS) and 2.5 mM D-glucose. The next day, cells were washed three times with KRBH containing 2% FCS and 2.5 mM D-glucose, and then incubated with 2.5 mM D-glucose followed by 16.7 mM D-glucose and 16.7 mM D-glucose plus 10 mM theophylline (37 °C, 0.5 mL/well, 2 h). The C-peptide concentration in the buffer was measured using the murine C-peptide ELISA kit, U-Type (Shibayaji Co.), which has a detection limit of 30 pg/mL The stimulation was expressed as the fold change of C-peptide concentrations in buffer compared with the low D-glucose treatment of the same well.

1. Reddy JK, et al. (1984) Induction and origin of hepatocytes in rat pancreas. *J Cell Biol* 98(6):2082–2090.

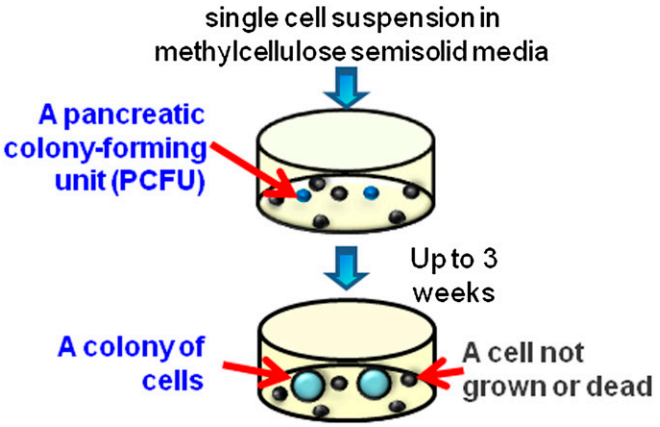

Fig. S1. Concept for in vitro methylcellulose-based colony assay. Pancreata were dissociated into single-cell suspension by collagenase B and DNase I and plated in semisolid media for colony formation. Methylcellulose is a biologically inert material that provides viscosity to the media, restricting the movement of single cells. A progenitor-like cell (i.e., a pancreatic colony-forming unit) may self-renew and differentiate and give rise to a colony. By analyzing lineage marker expression in a colony, the lineage potential of the originating single cell can be revealed. By dissociating and replating the cells from a colony, the self-renewal potential of the initiating single cell can be determined. Two pancreatic colony assays are established in this report; one contains Matrigel and the other laminin hydrogel.

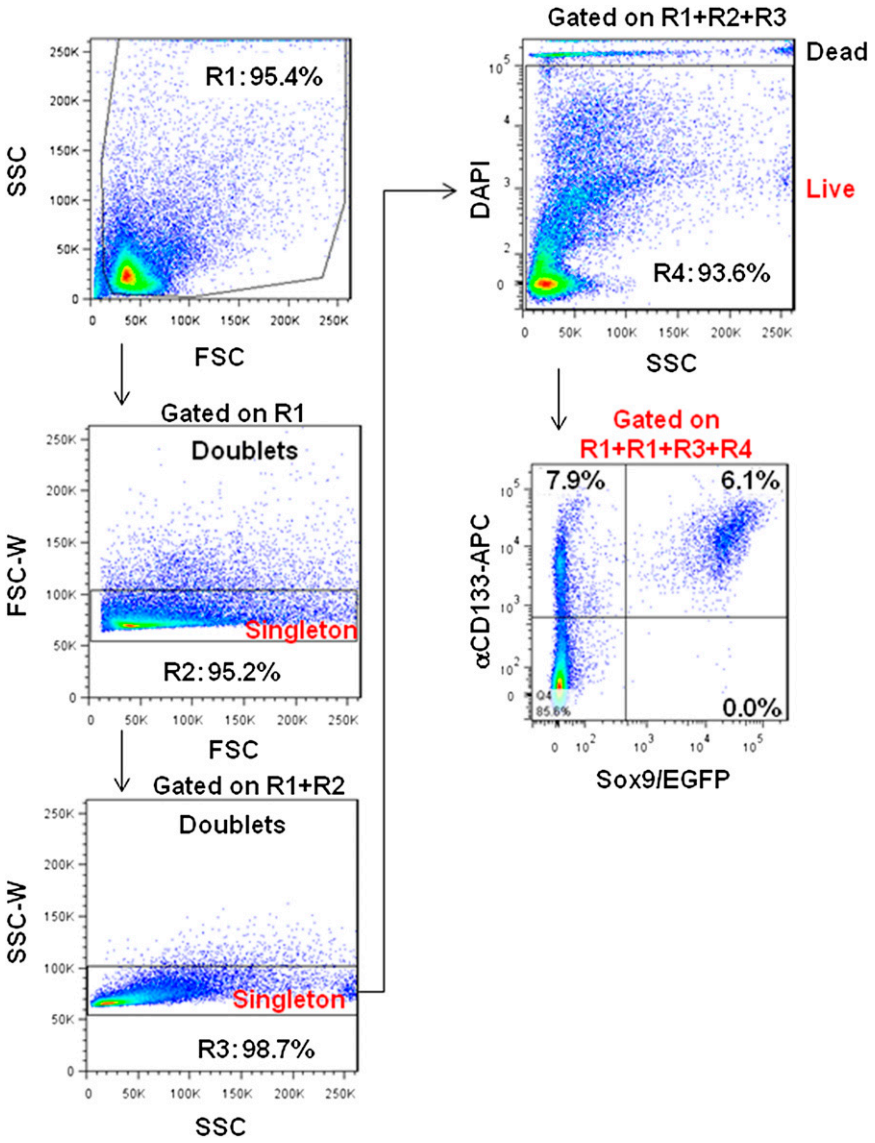

Fig. S2. Representative sorting windows for CD133^{+}Sox9/EGFP^{+} cells.

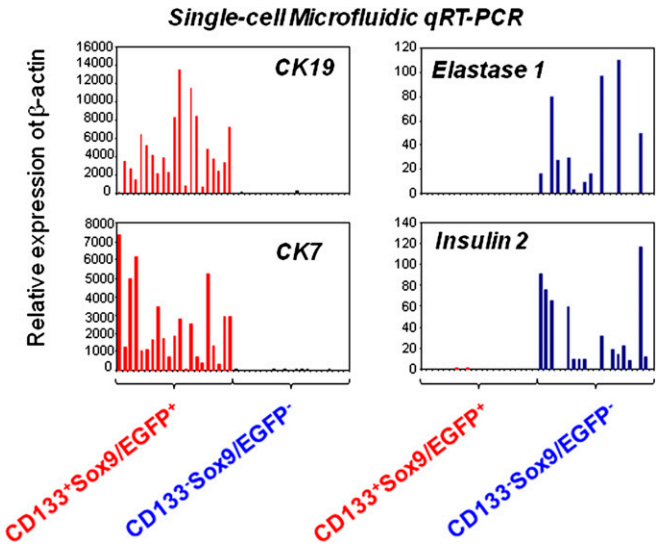

Fig. S3. Microfluidic qRT-PCR analysis on individually handpicked cells reveals that CD133^{+}Sox9/EGFP^{+} cells express ductal but not acinar or endocrine markers. Each bar is from a single cell.

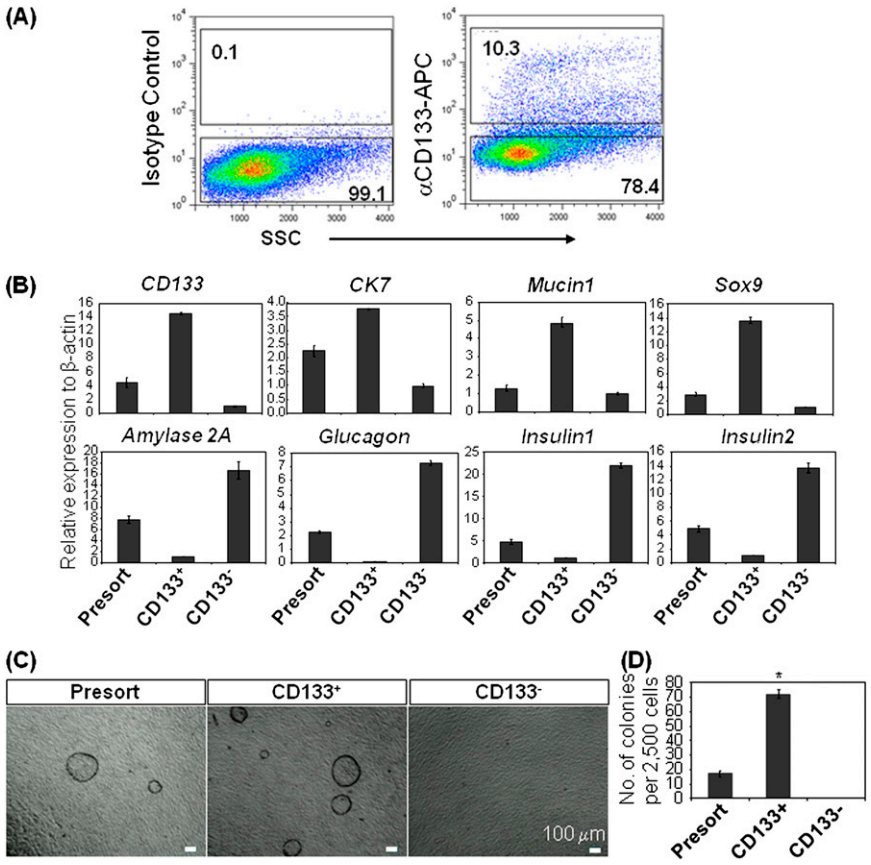

Fig. S4. Sorted CD133^{+} cells from adult B6 pancreata are enriched for ductal gene expression and Ring colony-forming activities. CD133^{+} cells were stained with anti-CD133 antibody and sorted by fluorescence-activated cell sorting. (*A*) Flow cytometric analysis of dissociated adult pancreatic cells. (*B*) Sorted CD133^{+} and CD133^{-} cells were analyzed by qRT-PCR for expression of pancreatic lineage markers using Taqman probes. (*C*) Photomicrographs of colonies grown from different sources of cell populations 3 wk postplating. (*D*) The number of colonies generated from each population of cells was scored. Data represent mean ± SD of colonies from triplicate wells. *$P < 0.05$ compared with the presort cells.

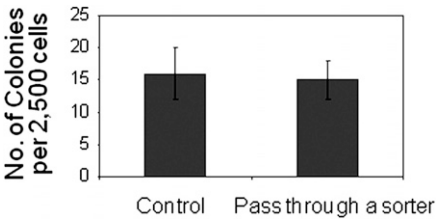

Fig. S5. Colony-forming efficiency of dissociated adult pancreatic cells that have passed through a sorter is the same as that of cells that have not (control).

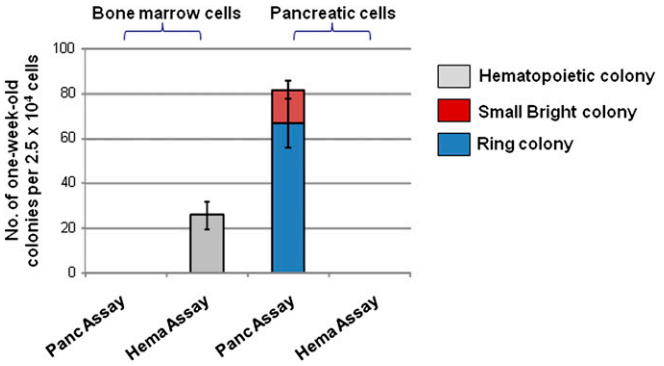

Fig. S6. Hematopoietic cells do not form Ring colonies in the pancreatic colony assay containing Matrigel. A total of 25,000 cells from bone marrow (femur) or pancreas were plated per well. Hematopoietic colony assay medium contains DMEM/F12, 1.0% methylcellulose, 30% FCS, 100 ng/mL interleukin-3, 100 ng/mL interluekin-6, and 2 U/mL erythropoietin. Colonies were counted 7 d postculture. Data represent the mean and SD of values obtained from three wells.

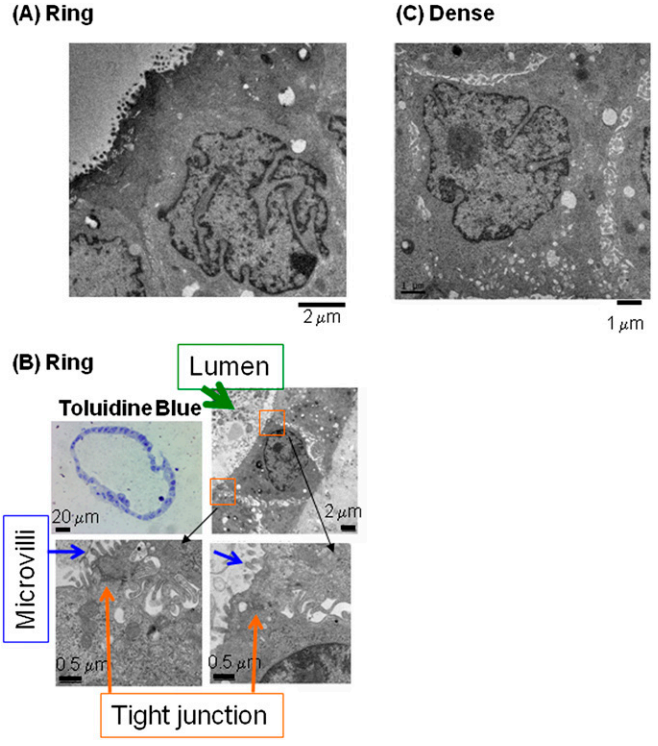

Fig. S7. Transmission electron microscopy reveals cells in 3-wk-old Ring colonies have ultra-structures consistent with ductal phenotype, including multilobed nucleus (*A*) and microvilli and tight junctions facing the lumen (*B*). (*C*) A 3-wk-old Dense colony also contains ductal-like cells.

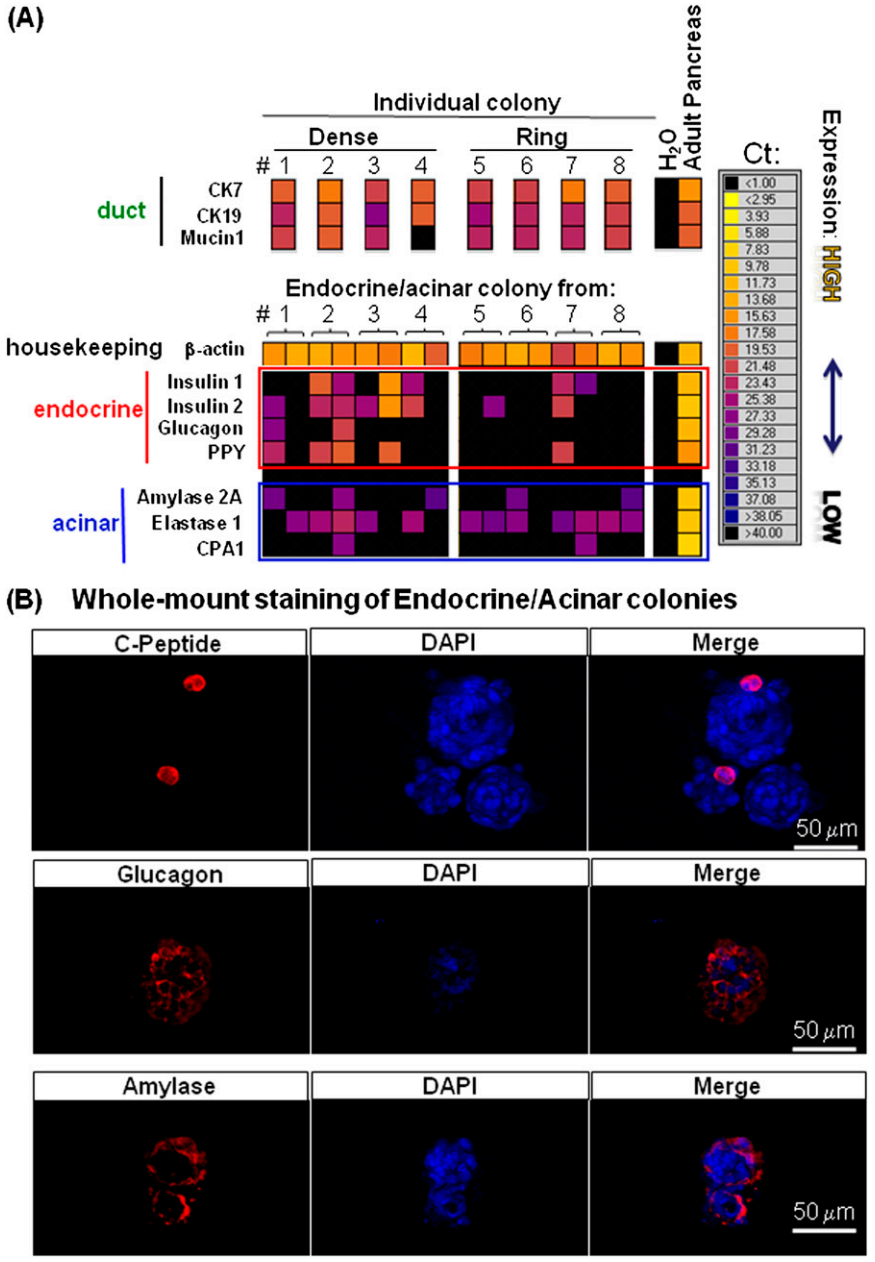

Fig. S8. Trilineage potential of single PCFUs–Dense. (*A*) Individual CD133^{+}Sox9/EGFP^{+} cells (n = 120 each group) were plated at 1 cell/well in a 96-well plate containing Matrigel and with (for Dense colony formation) or without RSPO1 (for control Ring colonies). Three weeks later, individual Dense or Ring colonies (n = 4 from each group) were handpicked and dissociated into single-cell suspension. One half of the dissociated cells was analyzed for expression of ductal markers (*Upper*), and the other half was replated into laminin hydrogel and cultured for 2 wk. Two of the resulting Endocrine/Acinar colonies from each well were randomly selected and analyzed by microfluidic qRT-PCR analysis (*Lower*). Each column is from a single colony. (*B*) Whole-mount immunostaining of Dense colony-derived Endocrine/Acinar colonies.

Fig. S9. Amino acid sequence of the artificial extracellular matrix protein used in this report. "Laminin" denotes a partial sequence from laminin α1 chain.

Table S1. List of murine Taqman probes used for microfluidic quantitative RT-PCR analysis

Murine gene	Assay ID from Applied Biosystems-Invitrogen
B-Actin	Mm 00607939_s1
B2 microglobulin	Mm 00437762_m1
Cyclophilin G	Mm01328875_m1
CK7	Mm 00466676_m1
Carbonic anhydrase (CA) II	Mm 00501572_m1
Mucin 1	Mm00449604_m1
Sox9	Mm 00448840_m1
CD133	Mm00477115_m1
C-Met	Mm01156972_m1
Insulin 1	Mm01259683_g1
Insulin 2	Mm 00731595_gH
Glucagon	Mm 00801712_m1
Pancreatic polypeptide (PPY)	Mm 00435889_m1
Somatostatin	Mm 00436671_m1
Ghrelin	Mm 00445450_m1
Amylase 2A	Mm02342487_g1
Elastase 1	Mm00712898_m1
Carboxypeptidase A (CPA) 1	Mm 00465942_m1
Ngn3	Mm00437606_s1
Pcsk1	Mm00479023_m1
Pcsk2	Mm00500981_m1

Table S2. List of antibodies used for immunohistochemistry

Antigen	Species/conjugation	Source	Dilution
Primary antibodies			
Sox9	Rabbit	Chemicon	1:1,000
Pdx-1	Guinea pig	Chris Wright (Vanderbilt University, Nashville, TN)	1:10,000
Glucagon	Mouse	Sigma	1:5,000
C-peptide	Rabbit	Cell Signaling	1:500
Amylase	Rabbit	Sigma	1:500
EGFP	Rat	Chrissa Kioussi (Oregon State University, Corvallis, OR)	1:1,000
Mucin-1	Armenian hamster	Lab Vision	1:200
Osteopontin (Spp1)	Goat	R&D Systems	1:1,000
Ngn3	Rabbit	Millipore	1:500
Secondary antibodies			
Rabbit/goat/mouse/guinea pig/rat	Alexa-488	Invitrogen	1:2,000
Rabbit/goat/mouse/guinea pig	Cy3	Jackson ImmunoResearch	1:2,000
Rabbit/goat/mouse/guinea pig/armenian hamster	Cy5	Jackson ImmunoResearch	1:500